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The Wesley M. Carpenter Lecture

IMMUNITY—GENERAL AND LOCAL

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I.

It is not my intention to recapitulate the fundamental facts of immunology, nor to discuss theories of mechanism. This would be mere repetition of conventional formulas with which you are familiar. Clinicians have begun to apply the reasoning technique of the laboratory to the study of therapy of infectious diseases. When, therefore, a specialist is permitted to discuss his subject before them, physicians are interested in knowing whither the trend of his subject is leading and in which directions it promises to progress beyond the understanding already attained.

Like other branches of knowledge, immunology has had its alternating periods of rapid advance and apparent stagnation. Ten years ago there seemed to be such a slowing down during which the discoveries of the pioneers were being consolidated and practically applied. During the last ten or fifteen years we seem to have broken into open country and the hounds are in full cry again; many of them, no doubt, on the wrong scent, but in other cases there is promise of fundamental change in immunological conceptions and practice. It is naturally impossible to do

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more than select, from a wealth of material, a few topics which appear to have particular bearing on clinical medicine, and into which my own work has led me.

Immunity in its broad connotation is the study of reactions of animal and plant tissues to foreign materials which can neither be utilized for nutrition nor excreted without alteration. It is obvious that the types of cellular and humoral responses, therefore, will be of many kinds, according to whether the foreign substances are soluble or insoluble, inert or toxic, dead or living. Between the giant cell formation occurring about cotton fibres or steel splinters, the cellular accumulations about tubercle bacilli or treponemata, or the complex structure of staphylococcus abscesses there are profound differences, but they are all expressions of a fundamental mechanism which protects the integrity of the tissues.

The various responses which are initiated by the entrance of foreign materials constitute inflammation, and this term—though usually applied to the local reactions—should be physiologically extended to include the deeper consequences which take place in remote regions of the body, influencing the distribution of leucocytes and perhaps the activities of other cells in distant parts. Conceived in this way, inflammation is the expression of natural immunity by which all animals and plants, not previously invaded, defensively respond.

The forces of natural resistance are normal reactions, a physiological emergency apparatus, called into play when foreign materials enter the tissues. When the invading substance, as in the cases of the cotton fibre, the steel splinter, or inhaled dust, is non-antigenic, the reaction accomplishes its purpose and the mechanism returns to normal quiescence essentially unchanged to future stimuli. With the so-called "antigenic" materials, however, repeated or prolonged contact leads to specific enhancement of the defensive reaction. And analysis of these increased defenses constitutes our knowledge of artificial immunity.

The fundamental fact of immunology is the existence of antigenic materials. The term signifies that this class of substances, once in contact with the tissues, arouses responses which specifically modify subsequent reactions. The antigens are all proteins and the antigenic property and its specificity are closely related to chemical structure. All native proteins which are amenable to enzyme action—that is, neither denaturized nor racemized—are antigenic. And infectious disease, on this basis, is nothing more than the presence in the body of a living and multiplying antigen which is sometimes extraordinarily insoluble, is often toxic and may frequently possess selective invasive power for special organs or tissues. The nature of the clinical disease is dependent upon the habitual portal of entry, upon the tendency for general or selective invasion, and upon the specific pharmacological action of poisons that may be produced. The nature of local lesions, also, may vary according to the solubility and the chemical constitution of the microörganism.

The chief difference, then, between natural immunity and acquired immunity to infectious disease is that which results from the antigenic properties of the bacteria and their poisons. The most obviously determinable factor in this difference is that of antibody production. And when we compare the reactions of a normal animal with those of a recently immunized one, the decisive differences between them are largely those dependent upon the activities of the circulating antibodies either in the prompt neutralization of toxins or in the accelerated destruction of the bacteria themselves. But antibody production is after all, only the expression of underlying cellular activities and immunity may persist after antibodies have disappeared. There remains in the immunized animal a specifically increased power to react with the responsible antigen. This expresses itself as hypersensitiveness which, with the non-bacterial antigens, may be entirely harmful. But hypersensitiveness to the bacterial antigens may, in some of its aspects, represent an acceleration of the local inflam-

matory reaction and, in this sense, possess defensive functions. Moreover, the increased specific reaction capacity is also expressed by an acceleration of antibody production. Whether, in addition to this, there is an occasional increase of local resistance to injury by the cells at the habitual portal of entry, as believed by Besredka, is not yet determined.

In regard to the mechanism of specific antibody production, we are quite as much in the dark as we have ever been. But there is a tendency in immunology to-day to question classical formulas like the side chain theory and to return to the original conceptions which favored the incorporation of some of the antigenic constituents in the antibody. Such a view takes its clue particularly from the fact that proteins which have become resistant to enzyme action are no longer capable of antibody formation, a condition which implies the necessity for at least a partial, probably cellular, digestion of the antigen in the course of the generation of the antibody.

Such problems, however, though of great fundamental importance, are as yet far removed from clinical interest. There are other phases of modern immunological investigation which have more direct bearing on clinical theory and practice. It is some of these that I wish to submit to you.

II.

Among the most interesting of these developments is that dealing with the non-specific enhancement of the natural defenses.

Natural resistance depends upon the coöperation of a number of different forces. Among these are the bactericidal powers of the blood plasma in which the alexin of Buchner is especially concerned but in which heat-stable bactericidal substances, less thoroughly studied, play an important rôle. Normal plasma also contains opsonic substances which lead to active phagocytosis by polymor-

phonuclear leucocytes. These forces are the ones most easily studied, but they are no more important, and perhaps subsidiary to the defensive activities of cells of the reticulo-endothelial system.

This system of cells, as defined by Aschoff, comprises cells widely distributed in the body, of probably similar physiological function, and recognizable, among other things, by their ability to take up intravital dyes. It includes the endothelial lining of blood and lymph capillaries, connective tissue elements, and particularly endothelium of the spleen, the lymph nodes, the liver capillaries, together with the mobile histiocytes, clasmatocytes and monocytes. One of the first to attribute protective functions to cells of this system was Kyes, who demonstrated the destruction of pneumococci by the Kupffer cells of the liver and the reticular cells of the spleen in naturally immune pigeons. Berry and Melick showed, later, that pneumococci are collected within these organs in a few hours and destroyed within a day. Especially convincing of the protective functions of the large mononuclear cells of this system are the investigations of Gay and his collaborators with streptococcus infections in rabbits.

Attempts to show parallelism between each of the protective forces mentioned and natural immunity have been numerous, but it is likely that all of them are active together and that with different infections in various animals the preponderance of one or the other process is specifically characteristic of the given case.

For purposes of discussing methods of enhancing natural resistance it is best to consider separately local natural resistance and systemic resistance.

Renewed interest in local resistance, both natural and acquired, has been revived by the work of Besredka. He believes that in various infections there is considerable difference in the susceptibilities of individual tissues. Thus, anthrax infection takes place easily in the skin, but, he asserts, if the organisms can be brought into the body

without injury to the skin, considerable numbers may be introduced without harm. In a similar way the bacilli of the enteric diseases and of cholera are conceived as having specific virulence for the intestinal mucosa. Besredka believes that it is sufficient in each particular case to immunize only the susceptible tissues and thereby prevent infection. From these considerations developed his methods of local immunization of the skin in anthrax and his vaccinations by mouth in typhoid, dysentery and cholera. The theory which he has built upon his experiments is a complicated one and will not be reviewed because we believe it to be untenable. On the other hand, some of his experiments, though in our opinion erroneously interpreted, have led to an understanding of many empirical methods for the treatment of local infections and have encouraged the development of new procedures for this purpose. In cutaneous infections with staphylococci and streptococci he found that prophylactic and some therapeutic effects could be achieved by compresses and injections of broth culture filtrates of the organisms causing the infections. His observations regarding the protective effects of broth compresses and injections have been corroborated by Gay and Morrison, by Rivers and Tillet and, in our own laboratory, by Mallory and Marble, but it has been found by these investigators that broth uninoculated with the organisms is quite as effective as are the specific preparations. Gay and his collaborators, who have analyzed these conditions with streptococcus infections in rabbits, have demonstrated that injections of broth into the pleura lead, within seventy-two hours, to a massive accumulation of clasmatocytes, and that at this period there is a considerably enhanced local resistance. Indeed, Gay has further shown that after an accumulation of macrophages has been stimulated in one area, the cells may be re-mobilized in adjoining areas by further broth injection or by infection.

If so simple a substance as a broth filtrate will produce a reaction that enhances the chemotactic accumulation of

protective cells, it would be surprising if this could not be brought about by many other stimuli, and—indeed—we seem here to have an experimental explanation for the non-specific empirical methods of the wet dressing, the compress, the poultice and numerous local effects which have hitherto been mysterious. Moreover, it is not impossible that the mechanism by which broth acts may be similar to that which is involved in massage, in the application of heat, and in the influence of light radiations. Cramer, who has studied the effects of X-rays, regards radio-therapy as in part a stimulation of the reticulo-endothelial tissues, and correlates the effects of such treatment with other non-specific agencies that can increase natural resistance. Obviously, in these simple and, in some cases time-worn procedures of minor surgery, we have methods which accelerate the inflammatory protective mechanism which is set in motion by the infection itself—but perhaps with insufficient vigour and possibly is slowed down by coincident toxic injury.

It is of course difficult to come to any conclusions about this unless we can learn more of the forces which induce local inflammatory changes and cellular accumulations. This subject is obscure, but a plausible point of view has been brought forward by E. F. Müller. Müller's point of departure was an observation made in the course of the non-specific therapy of chronic urethritis. In such cases it was observed that intramuscular injections of 10 or more c.c. of lactalbumen, an entirely non-irritating substance, was followed within three to five hours by noticeable aggravation of the chronic inflammation, followed by an increase of secretion and the appearance of living leucocytes in the exudate. While amounts of more than 7 c.c. of the lactalbumen were necessary to produce this effect intramuscularly, the same result could be achieved with amounts as low as 0.1 to 0.2 c.c. injected intracutaneously. Müller found that within a short time after the injections there was a determinable change in the distribution of leucocytes, the immediate effect being a sharp drop of the leuco-

cyte counts in the capillary blood. This sudden and considerable diminution of the capillary leucocytes, unaccompanied by corresponding changes in the blood of the larger vessels, implied a corresponding accumulation of leucocytes in the viscera. Since the effect could be brought about by the intracutaneous injections not only of lactalbumen but of other non-irritating substances like distilled water, Müller concludes that the general effect is secondary to a reflex stimulated in the sympathetic nervous system. He believes that injections into the skin initiate, through the autonomic nervous system, a peripheral vaso-dilatation with corresponding vaso-constriction in the splanchnic areas, and that the alteration in leucocyte distribution depends upon forces secondary to the changes in the vascular walls. The dependence of alterations in leucocytic distribution upon vaso-motor conditions had previously been emphasized by physiologists—Garrey among others. By referring such reactions to impulses stimulated in the sympathetic nervous system, Müller makes it comprehensible how such entirely unrelated skin stimulations as hot packs, wet compresses, radio-therapy, Bier's therapy and massage can result in similar phenomena. We are of course not yet ready to accept Müller's results, since as far as we know they have not been experimentally confirmed.

Incidentally, these observations, together with related experience from the field of hypersensitiveness, strengthen a growing impression that we must look upon the skin as a special organ, with individual functions and reaction capacities considerably more far reaching that its mere significance as integument.

While we do not share the extreme views of Besredka regarding the strict limitations of susceptibility and immunity in given diseases to specific tissues, available information indicates that, together with a systemic general immunity, there is often a relatively more intense immunization of those cell complexes that have been in direct conflict with the invaders. This is quite obvious in

staphylococcus and streptococcus (erysipelatous) infections of the skin; and, in rabbit syphilis, we have had evidence of this in our own experiments. It may be true of the intestinal mucosa as well, after enteric infections—but of this no adequate proof has been adduced. That a certain degree of immunization can be obtained by vaccination is likely, but there is no evidence that this is not due to a systemic rather than local immunization and, at best, the results are too irregular to be substituted for the more easily controlled prevalent methods.

Immunologists, fifteen years ago, were so deeply under the spell of ideas of specificity that it required considerable accumulations of evidence to induce them to give serious attention to the non-specific enhancement of systemic resistance or what is spoken of as "non-specific protein therapy."

We must accept as a fact that the injection of many substances—particularly bacterial proteins, foreign sera, etc.—may exert a profoundly beneficial, sometimes curative effect upon a variety of acute and chronic infections, provided dosage and activity of the chosen substance are appropriate to the physiological responsiveness of the patient. By this we mean that too large a dose may be as harmful as an appropriate dose may be beneficial, and that a material and dosage likely to give excellent results in a vigorous patient may perhaps be harmful in a patient already considerably enfeebled. It is, therefore, a therapeutic procedure not without danger, but it cannot be ignored as an important field of study. Petersen, Weichardt and many others have endeavoured to analyze the processes which are set in motion by such injections, both in the normal and in the infected body, and investigation has shown that it is quite impossible to attribute the non-specific elements of these reactions to any simple scheme. As Weichardt expresses it: "However attractive it may be to refer the phenomena of protein therapy to a single principle, it is nevertheless quite definite that we are confronted with a very complex train of events, in which chemical, physical

and physiological occurrences are inter-related." To mention only a few of the significant things which follow upon the injection of—let us say—typhoid vaccine in moderate dose: There is a powerful reaction in the bone marrow, with temporary leucopenia followed by a considerable increase in circulating leucocytes; there is enhanced activity of the reticulo-endothelial system and an increase in blood platelets; there is temporary speeding up of metabolism, an increased concentration of blood enzymes, and an increase of fibrinogen and blood globulin; there is a profound change in the relative distribution of blood in the visceral and splanchnic areas, with consequent effect upon leucocyte distribution, and with the development of chills and fever, according to alternating peripheral or visceral vaso-constriction. Müller and Petersen made the significant observation in this connection, that the injection of either bacteria or peptone gave rise to reflex vaso-motor change in which there was a general vaso-constriction on the periphery and a simultaneous vaso-dilation of the splanchnic vessels. This may well explain the severe chills we have all noticed following accidental intravenous inoculation of typhoid vaccines, and it is not impossible that the accompanying reactions of cells and tissues may be secondary to this powerful vascular stimulation. It is reasonable to regard the general effects in these phenomena as analogous to a sort of systemic inflammatory reaction, in which there is a temporary increase of all the physiological activities, and among them an enhancement of the defensive mechanism.

In addition to the purely non-specific effects, such protein injections seem also to stimulate any existent latent specific capacities. Animals that have once been immunized and allowed to rest until antibodies have disappeared from the circulation, may again produce antibodies when injected with non-specific substances such as salt solution, nucleic acid, etc., and this is probably the basis of some of the successes reported in sub-acute and chronic conditions by such injections. This would explain, among other

things, the partially specific effects when the method is applied in the course of diseases such as typhoid fever at times when antibody formation is initiated but perhaps lagging.

III.

As a natural corollary to studies on methods of reënforcing natural resistance there has developed a growing interest in problems of host susceptibility. There are many infections to which man is so highly and uniformly susceptible that practically all previously uninfected individuals contract them if thoroughly exposed. Thus there are no significant differences in human susceptibility to measles, mumps, smallpox, influenza, cholera, plague and some other diseases, and the supposedly greater susceptibility in childhood can be shown to be fictitious and attributable to the fact that exposure and consequent immunization is sure to have occurred before adolescence.

There is another class of diseases like the common cold and pneumonia in which the average resistance of the normal human being is relatively high and in which infection occurs, as a rule, in the train of predisposing diseases or of fatigue, exposure to sudden changes of surface temperature and other accidental depressing factors which let down the bars that ordinarily prevent penetration.

The fluctuations of normal susceptibility, however, which interest us most are those in which there seem to be distinct systemic differences in previously uninfected people which are not dependent merely upon resistance-depressing accidents.

Among these factors of difference the ones most actively discussed have been what Draper and others have spoken of as "constitutional types," and the influences which may be exerted upon susceptibility and resistance by nutrition, particularly as regards vitamin deficiencies.

The constitutional type concept is an interesting one,

but extraordinarily difficult of experimental approach. The nutritional factors are more easily studied.

The disease in connection with which this type of relationship has been most considered is tuberculosis, in which it is well known that nutritional disturbances predispose, whether they are due to faulty diet and digestive difficulty, or to the nutritional consequences of debilitating acute or chronic disease. There has been much evidence in the post-war increase of tuberculosis in certain European countries which has suggested direct relationship between the incidence of tuberculosis and fat deficiency. The time-honoured use of cod liver oil may depend upon its fats—or more probably upon its vitamins—but there seems to be little doubt of its actual beneficial influence. In spite of this, we have little conclusive experimental evidence which would give the conception greater precision, except perhaps the experiments of Lange and Simmonds, which seem to show that rats fed on high fat diets, although deficient in vitamins threw off moderate tubercular infections with greater ease than did the controls.

The suggestion of dietetic relationship to susceptibility is not a recent one. Theobald Smith in 1913 attributed a stable epidemic among guinea pigs to a lack of green feed. The vitamin idea was not drawn into the discussion until later, and is beginning to accumulate a considerable literature, which is suggestive, though not yet conclusive. A number of investigators—Coulaud, Schilf and others—have obtained results differing from those of Lange in that they attribute a hastening of death in quinea pigs from tuberculosis to a lack of vitamin C. Gloyne and Page, who studied tuberculosis in rats fed on a vitamin A-free diet, found that the animals died before the controls from an intercurrent pneumococcus infection, rather than from tuberculosis, and in many similar experiments of other investigators this same increased susceptibility to secondary infection has been noticed.

A number of studies have dealt with the predisposing in-

fluence of scurvy. Abel's article of 1924 emphasized the importance of the early stages of scurvy during which, without marked scorbutic symptoms, increased susceptibility to a number of different infections—including such dissimilar things as influenza, tuberculosis and vaccinia —was noticed.

The most important investigations dealing with this question are those in which susceptibility to spontaneous infections in animals were studied, since in such delicate balances as the ones involved, experimental infection represents too severe a test for slight differences. For this reason, there is particular weight in the experiments of Webster and his associates, who observed greater resistance on the part of mice fed on McCollum's diet, both against mouse typhoid and against botulismus toxin. Similar to this work are the experiments made in 1928 by Schmidt-Weyland and Koltzsch. Taking advantage of the frequent epidemic respiratory infections occurring in guinea pigs, they attempted to infect animals through the respiratory passages with sprays of broth cultures of pneumococci and of chicken cholera bacilli. Other animals they infected with the same material by feeding. Comparing the results obtained in normally fed animals with those in animals subjected to scorbutic diets, they not only determined a much higher susceptibility to intentional infection among those suffering from scurvy, but obtained in these animals a much higher percentage of accidental infections.

That nutritional disorder predisposes to infection seems likely, therefore, from clinical and experimental data. But it has not been easy to attribute such change to any specific vitamin factor. Regarding the effect of vitamin A, most of the work that was reported until recently has been contradictory. An exceptionally clean-cut result, pointing to the importance of vitamin A, is the recent study of Green and Mellanby, who found that rats fed on diets deficient in vitamin A developed marked susceptibility to pyogenic infections not noticed in control rats. Prompt feeding of vitamin A often resulted in cure, with disap-

pearance of the infection. Other studies that seem fairly consistent appear to attribute considerable importance to vitamin B. Thus Werkman found that rats suffering from lack of vitamin A or B become more susceptible than the normal to anthrax, and that pigeons fed on a low B diet become susceptible not only to anthrax but to pneumococcus. Similar observations were made on pigeons with low vitamin B diets by Findlay, who further determined that such pigeons become susceptible to Gram negative micrococci to which they are normally entirely resistant.

It is apparent from this brief account that the subject is still in much confusion. Nevertheless, enough has been done to attract the immunologist to the study of nutritional conditions, especially in efforts to explain the quite remarkable irregularities with which some infectious diseases—either sporadic or epidemic—select certain individuals of a group who present no obvious differences from others equally exposed.

Irregularity in susceptibility is particularly marked in some of the diseases caused by neurotropic ultra-microscopic viruses. We are referring particularly to infantile paralysis and the various forms of encephalitis. In poliomyelitis the matter has given much cause for thought to a number of investigators, and it is particularly Draper and Aycock who have expressed the view that the disease has a tendency to occur in individuals of certain constitutional types, and Aycock has tentatively endeavoured to correlate this with what he calls an "imbalance" of growth and metabolism due to increased or insufficient activities of the internal secretions, possibly influenced by seasonal fluctuation.

Our own interest in the matter was chiefly derived from a continued study of herpes encephalitis. Febrile herpes, the common cold sore, is obviously secondary to some direct or indirect tissue injury initiated either by irritating secretions, sunburn, etc., or systemically by certain febrile diseases, and even by vaccine injections. The virus derived from herpetic vesicles can produce in rabbits, guinea pigs, rats and certain species of monkeys an en-

cephalitis which has certain clinical and epidemiological similarities to encephalitis in man. It is a strikng fact that certain forms of encephalitis in man are secondary to predisposing diseases, the most important among which are influenza, measles, chicken pox, smallpox and vaccinia. And since of the many who suffer from the predisposing diseases a few only develop encephalitis, the conclusion is inevitable that an individual susceptibility factor is involved.

We do not agree with Levaditi that there is adequate evidence to prove that herpes virus is the cause of human encephalitis. In his later papers no new facts are brought forward to reënforce earlier opinions. We are not even sure that all forms of encephalitis are infectious rather than toxic, but the similarity of encephalitic lesions with those of truly infectious conditions and the fact that most of the diseases that predispose are filterable virus infections inclines us to hold the view also expressed by McIntosh and others, that many filterable agents may become neurotropic when the unknown susceptibility factor exists. Herpes virus is an excellent material with which to study these relations for, as we have found, both in Cebus monkeys and in rats there is a natural difference in individual susceptibilities analogous to that in man. In Cebus monkeys we have been able to produce practically all the clinical varieties of encephalitis that occur in man, from the acutely fatal to the chronic, and some of the animals appeared to be entirely immune. Efforts to correlate this with nutritional conditions have not been conclusive, because in animals with extreme malnutrition it is difficult to differentiate between the neurotropic effects of the diet and minor lesions of encephalitis. In several series of rats a vitamin-free diet increased susceptibility from an average of 55 per cent to one between 90 per cent and 100 per cent, but here again it was necessary to carry the dietetic injury to such extreme degrees that no significant conclusions could be drawn. Further experimentation along these lines, however, is being continued.

In our studies with typhus fever, a disease which is notoriously tempestuous in times of famine, we have found that guinea pigs and rats in the late stages of scorbutic diet effects react much more severely than do normal animals, and in the rats there is a considerably greater than normal accumulation and distribution of the Rickettsia organisms which we feel confident are the causative agents of the disease.

Though the influence of diet and vitamin deficiencies offers for the moment an experimental approach more accessible than that of the constitutional difference, it may well be that dietetic deficiencies like debilitating disease or preceding toxic injury should be regarded merely as indirect influences bearing upon physiological activities of another kind. I have presented the subject in the full knowledge that it is still quite vague and speculative, but with the purpose of illustrating directions of thought in immunology which are of the greatest importance and to which experimental groping of the kind outlined is the only procedure which can eventually bring enlightenment.

IV.

No investigations have had a more far-reaching effect upon immunology than those dealing with bacterial dissociation. Much that was mysterious in the past regarding differences in antibody production when animals were immunized with non-virulent, heated or otherwise altered bacteria, has become clear. It has been shown that bacteria may assume a number of mutation forms visibly demonstrable by morphological alteration or by changes in manner of growth. What is most important, however, is the fact that with such obvious changes there is correlated a fundamental biological one which affects both virulence and the structure of the antigen.

Arkwright, who was one of the pioneers of the modern development of this subject, applied the words "rough" and "smooth" to the two types of organisms obtained from both

dysentery and typhoid cultures, because of the appearance of their respective colonies on agar plates. The dissociation of several mutation types has since then been accomplished with a majority of the pathogenic organisms, and it has been found that in most cases "smoothness" or the "S" type is associated with virulence, and "roughness" or the "R" form with relative loss of virulence. Dissociation is not limited to two types only, there may be intermediate stages between the completely virulent and the relatively harmless forms, and there are, in the motile organisms, where the flagellar substance forms a separate antigenic complex, four types—as studied by Li in hog cholera and by Grinnell in typhoid—motile rough, motile smooth, non-motile rough and non-motile smooth forms.

The antigenic modifications which accompany the morphological changes have profound bearing on clinical immunology. We may summarize them by stating that, in the non-motile groups like the streptococcus, pneumococcus, and possibly meningococcus and other organisms, the chief difference between a fully virulent and a so-called "rough" organism consists in the fact that the smooth or virulent contains the complete antigen in which there are, loosely combined, at least two substances—a nucleoprotein fraction and a carbohydrate material. Animals and man immunized with such "complete" bacterial antigens react with production of type specific antibodies. The carbohydrate material which carries the specificity is a haptene in Landsteiner's sense: it can combine with formed antibody, but cannot—dissociated from its protein fraction—induce antibody formation. This substance, which we identify roughly with the capsular or ectoplasmic material, is easily dissociated from its protein mate, is free in exudates or circulation, and by uniting with antibody and diverting it from the organisms interferes with bacterial destruction, thus contributing to the virulence of the bacteria, perhaps representing the so-called "aggressin" substance of Bail.

Since it is only the combination of the antigenic frac-

tions which can lead to the type specific antibody formation, the rough organisms—which contain only the protein constituents—though also antigenic, produce antibodies which are not type specific, but have in most organisms—certain streptococci excepted—a wider group specificity. It is the generally held opinion that these nucleoprotein antibodies have no particular protective significance. It is our own belief that the nucleoprotein antigen is related to allergic reactions and has in this sense an important pathological and immunological bearing. This, however, is neither fully determined, nor is it pertinent to the present discussion.

In the motile organisms such as the typhoid and paratyphoid groups, the complete antigen of the organism is represented not only by the two factors—protein and carbohydrate—mentioned but, in addition to these, by another antigen, complete in itself and functionally separate, which is present in the flagellar substance.

In toxin-forming bacteria like the diphtheria bacillus, roughness and smoothness expresses itself in capacity for toxin production, the toxin produced by the smooth types representing a separate antigen, not produced in cultures of the rough mutation forms.

It is obvious that these studies must profoundly influence clinical problems of vaccine treatment and serum production. Much of the older literature on different methods of growing organisms, different degrees of heat, or different kinds of chemicals to be used in vaccine production has become obsolete. We can now lay down briefly the necessary criteria for the preparation of the specifically effective vaccine. In view of the knowledge that most pathogenic species contain a number of serologically heterologous types, the original organism must be one homologous with that causing the condition to be protected against or to be treated. The organism used must be of the smooth, virulent variety, containing the whole antigen and all the antigenic constituents possessed by the

mutation form which causes the disease. It would be valueless to make a vaccine from a rough culture, and experiments of Li and the recent ones of Grinnell are showing that vaccines of the typhoid-paratyphoid groups are fully potent only when produced with the smooth organisms. In addition to the choice of organism, conditions and time of cultivation and further manipulation must be such that the effective antigen which gives rise to the preponderating protective antibodies is neither altered nor destroyed. Therefore, autolysis must be prevented, a condition which limits the choice of chemicals; also heating during sterilization may produce modifications. It appears from Grinnell's yet unpublished studies that even moderate degrees of heat may modify typhoid antigen, and, ordinarily, heat-killed typhoid suspension is considerably less potent in antibody production than a formalinized antigen. The antigenic effectiveness of formalinized bacteria has been reported by others and has been determined by us with pneumococcus vaccines; and in formalinizing capsulated anthrax bacilli we have found that complete morphology can be preserved by this method during many weeks of preservation. Formalinization requires more study in connection with vaccines for human use, and better methods may be evolved. In this place we wish merely to emphasize the criteria of vaccine production, which can now be simply formulated on the basis of mutation studies.

In regard to antitoxin production, the studies of our pupil Ho Yü, corroborating and extending those of other workers, confirm the necessity for periodical plating and renewed selection of smooth, toxin-forming organisms in order to keep toxin potency up to standard.

It is easy to modify a virulent S organism into the R form. This often occurs spontaneously in the course of cultivation on artificial media, and is an accompaniment of developing saprophytism. The process can be hastened by cultivation in bile broth, or in dilutions of inactivated homologous serum. This has epidemiological importance.

It has been shown that R pneumococci may appear in patients recovering from pneumonia, and our student Ho Yü has demonstrated that in convalescence from diphtheria R forms gradually increase in number in the throat, a process by which the carrier of virulent bacilli is often converted into a carrier of non-toxin-forming organisms. Since the R forms can be produced by cultivating toxin-forming S forms in antibacterial diphtheria serum, but not in antitoxic serum, this implies a possible solution for the difficult problem of the chronic diphtheria carrier, suggesting immunization of such individuals with vaccines made of dead, smooth, diphtheria suspensions. For it is quite possible that because of the purely local and superficial situation of the diphtheria bacilli, there is an insufficient absorption of the unchanged S bacterial protein to stimulate the natural formation of adequate amounts of the antibacterial antibodies.

The possible transformation in the body of S into R forms may therefore be significant in explaining the hitherto obscure fact that in many epidemics—meningitis, the pneumonias, typhus fever and some other diseases—the mortality and the speed of numerical case incidence go hand in hand. Not only do morbidity and mortality often show a parallel rise, but after the peak—when cases are relatively few—the mortality is also lower. A reasonable explanation for this might be that while transmission is relatively rapid in a large reservoir of susceptibles there is rapid transfer of virulent S forms from early cases to new victims; whereas toward the end of an epidemic, with fewer susceptibles, the average transfer to the new victim is at a much later stage of the disease, when virulence (S forms) has been diminished by longer contact with the body of the partially convalescent.

No less important for medicine and epidemiology is the observation that under special conditions a reverse conversion from R into S forms may be brought about. Levinthal and Dawson have shown that R forms may revert completely to the original virulence and specificity; and Grif-

fith has succeeded, by injecting mice with R forms of one type, together with considerable amounts of heated S culture of another type, in producing a transformation of a rough of the original type into an S of the type corresponding to that from which the heated S material was derived. Reimann has confirmed this, and Dawson has further succeeded in producing such conversion of one type into another in vitro. When he inoculated a type II R culture into tubes containing type III S substance together with blood broth and 10 per cent of anti-R serum, his R organisms were converted into type III S. This conversion from one type S into another type S never takes place directly, but only through the R mutant of the original type. The practical consequence of such transformation of nonvirulent into virulent forms, even without type alteration, is obviously of great possible importance in connection with the sudden sporadic occurrence of disease, and with the often mysterious origin of epidemics. It is especially significant in connection with influenza and cholera epidemics, perhaps explaining the unexpected development of outbreaks in regions where there had been no obvious pre-existing focus.

V.

In a sense, immunology is beginning over again. The principles that govern resistance to bacterial infection in general are applicable to a limited degree only in tuberculosis and syphilis, and are still less so in the protozoan diseases and infections caused by ultra-microscopic filterable agents. Each of these constitutes a special chapter which must be separately considered. We wish merely to point out that one of the developments of modern immunology is the recognition of a number of variants of the defensive mechanism about which we cannot generalize from our knowledge of immunity in the bacterial diseases.

The special case which we wish to discuss in some detail is that of the ultra-microscopic or filterable virus diseases,

because of our own interest in these problems, and because of the importance of these diseases in clinical practice.

In practically all of the conditions caused by filterable agents, an attack is followed by an immunity which—if not permanent—at any rate lasts for a number of years. Analysis of the circumstances attending such acquired resistance reveals a number of outstanding facts which differentiate this type of immunity from other types.

Important among these is the observation that active immunization can not be achieved with killed virus. For the establishment of resistance it seems necessary that the cells react with the living agent. Evidence based upon the presence of inclusion bodies and the virulent nature of washed cells of infective tissue indicate that the virus must enter the cell to cause disease. And if this is correct, it would explain why immunization can be attained only under conditions which permit such penetration. Acceptance of this principle would restrict our prospects of prophylactic vaccination in diseases like poliomyelitis, etc., to the discovery of methods of safe attenuation comparable to Jennerian vaccination in smallpox.

There are two apparent exceptions to the necessity of using living virus for prophylactic purposes. One is the Semple method of rabies prophylaxis with phenolized virus; the other, the formalin distemper immunization of Dunkin and Laidlaw. The exceptional character of these observations, and the necessity for delicate adjustment of the formalinization in the distemper experiments justifies some doubt whether the materials in these cases are killed rather than profoundly attenuated. Yet the observations imply practical consequences of such importance that they cannot be ignored. And it is possible that a sufficiently massive immunization with dead virus might give results. In another type of disease—Rocky Mountain spotted fever and the typhus group—in which similar conditions exist, encouraging results are being obtained with carbolized and

with formalinized virus, provided sufficient concentrations of the infectious agent can be obtained in the vaccine.

Examination of the conditions which determine immunity in a convalescent or in an animal immunized with a living agent has revealed that the serum contains substances which neutralize the virus in vitro. Incubation of the agent with such serum before injection often renders the virus innocuous, though in certain experiments of Andrewes with virus III and with vaccinia this was reliable only with skin injections and incompletely so when injections were made into brain or testis. In our own observations with herpes virus, neutralization in vitro was successful, but no passive immunization could be accomplished when the serum was introduced separately from the virus, even when serum preceded inoculation.

The protective serum body is not an antibody in the usual sense. It persists in the blood of convalescents for years, and it cannot be increased by hyper-immunization to anything like the extent that this can be done with bacterial antibodies. The manner of action of the protective bodies has been considerably elucidated by tissue culture carried out by the Maitland method. Andrewes found, with virus III and vaccinia, that if tissue and immune serum are mixed and then inoculated with virus, the agent does not multiply, and no inclusion bodies are formed. But if virus and tissue are brought together for only a short time, and then immune serum is added, the virus survives, and inclusion bodies form—even if the tissue has come from an immune animal. The experiments of Rivers are similar, except that he found a certain degree of independent resistance on the part of the immune tissues. Both of these observers were able to show that even in supposedly neutralized mixtures, the virus is neither killed nor firmly united, but can be recovered in virulent form.

These observations, as Andrewes points out, indicate that the protective bodies probably act merely in preventing the virus from entering the cells, but are not able to

affect the virus after it has become intracellular. Immunity therefore depends upon the preliminary presence of protective bodies in the fluids about the cells, and perhaps, to a limited degree, upon some cell resistance to injury.

Such a conception accounts for the difficulties of serum therapy with convalescent serum. If we reason from our own herpes experiments, it would seem that even large amounts of protective serum injected into the veins and into the cisterna magna before intracerebral inoculation confer absolutely no protection. Yet this experimental test is a severe one, and treatment with protective serum might well prevent extension from a local area into new cell groups. This is the theoretical basis for serum prophylaxis and for optimism in the limiting value of convalescent serum early applied in poliomyelitis.

There is another fundamental observation in the immunology of these conditions which must influence future investigation. In experiments of Andrewes with virus III and vaccinia, he succeeded in demonstrating that virus persisted, and can be recovered by filtration and dilution from over-neutralized mixtures. Rivers and his associates have shown that vaccine virus can survive for some time in preparations of immune cornea in anti-vaccinal plasma. There is much evidence that filterable agents can survive after recovery. This has been observed by Bang and by Loeffler in foot and mouth disease; it has been reported in contagious epithelioma of fowls, in the salivary gland virus of guinea pigs; and Olitsky and Long have succeeded, by cataphoresis, in separating vaccinia virus from the tissues of rabbits long recovered from cutaneous vaccination. There is indication here that the state of immunity may be related to persistence of the virus. A similar state of affairs has been generally assumed until very lately for syphilis, and it apparently exists also in protozoan diseases. There is here a problem of fundamental importance, since it implies necessity of a continued antigenic stimulus for a prolonged state of resistance.

Incidently, this persistence of the filterable viruses in tissues after recovery suggests an explanation for the frequent recurrence of herpes, in one and the same place, the virus persisting in the recovered tissues and regaining capacity for cell injury under the influence of local irritation by secretions, sunburn, etc.

I have attempted to give you an account of some of the more important problems that are occupying immunologists at the present time. I have been forced by the nature of my task to confine myself to the bare outlines of my topics. I hope that I may have succeeded in pointing out the possibilities of immunological investigation, and in showing that of all the biological branches of medicine no other is more deeply involved with the problems of the clinic.